Conversion of Oils to Monoglycerides by Glycerolysis in Supercritical Carbon Dioxide Media

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ABSTRACT: Glycerolysis of soybean oil was conducted in a supercritical carbon dioxide (SC-CO_2) atmosphere to produce monoglycerides (MG) in a stirred autoclave at 150–250°C, over a pressure range of 20.7–62.1 MPa, at glycerol/oil molar ratios between 15–25, and water concentrations of 0–8% (wt% of glycerol). MG, di-, triglyceride, and free fatty acid (FFA) composition of the reaction mixture as a function of time was analyzed by supercritical fluid chromatography. Glycerolysis did not occur at 150°C but proceeded to a limited extent at 200°C within 4 h reaction time; however, it did proceed rapidly at 250°C. At 250°C, MG formation decreased significantly ($P < 0.05$) with pressure and increased with glycerol/oil ratio and water concentration. A maximum MG content of 49.2% was achieved at 250°C, 20.7 MPa, a glycerol/oil ratio of 25 and 4% water after 4 h. These conditions also resulted in the formation of 14% FFA. Conversions of other oils (peanut, corn, canola, and cottonseed) were also attempted. Soybean and cottonseed oil yielded the highest and lowest conversion to MG, respectively. Conducting this industrially important reaction in SC-CO_2 atmosphere offered numerous advantages, compared to conventional alkali-catalyzed glycerolysis, including elimination of the alkali catalyst, production of a lighter color and less odor, and ease of separation of the CO_2 from the reaction products.
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Glycerolysis is carried out at industrial level to form monoglycerides (MG) from glycerol and triglycerides (TG). Different aspects of this complicated reaction, which were studied extensively in the 1940s and 1950s, have been reviewed by Sonntag (1). The reaction of TG with glycerol to yield partial glycerides is normally done at high temperatures (250°C) to increase the solubility of glycerol in the oil phase, which is only about 4% at room temperature. Alkali catalysts, such as NaOH, KOH, and Ca(OH)$_2$, are used to accelerate the process. At the end of the reaction time (usually about 4 h), the catalyst is neutralized, and the reaction mixture is cooled rapidly. This step is crucial to minimize reversion of the reaction, because glycerol separates into a heavier, lower layer upon reduction of temperature.

The resultant product is a mixture of MG, diglycerides (DG), and TG, as well as free fatty acids (FFA) and their metallic soaps. The MG yield is generally rather low at 30–40%, and the reaction product is distilled in a molecular still to achieve MG concentrations of about 90%. These MG and their derivatives are important emulsifiers for food, pharmaceutical, and cosmetic industries (2). Glycerolysis is also conducted as the preliminary step for the production of alkyd resins and some detergents, and the reaction is carried out on a considerable scale in various industries (3). Unfortunately, the high temperatures involved in the reaction often result in a dark-colored product. In addition, the presence of FFA and their metallic soaps can lead to associated flavor and odor problems in the food products into which they are incorporated. The utilization of solvents that can solubilize both oil and glycerol to decrease the high reaction temperatures also has been investigated (1). However, few solvents (phenols, cresols, 1,4-dioxane, and pyridine) promote the desired miscibility, and these are not acceptable in trace amounts when MG/DG mixtures are compounded in food products.

Over the last decade, lipase-catalyzed glycerolysis under milder reaction conditions, has received increasing attention in an effort to minimize the abovementioned flavor, odor, and color problems associated with conventional glycerolysis. For example, MG have been prepared from corn oil (4) and tallow (5) with immobilized lipases. By keeping the temperature below a specific level (30–46°C), saturated fatty acids could be concentrated in the MG fraction during lipase-catalyzed glycerolysis of palm oil (6), beef tallow, and lard (7). MG yields as high as 90% could be achieved with incubation times up to four days (8,9). A membrane bioreactor also has been used for the glycerolysis of olive oil, but MG yields were low (10). However, use of an in-line adsorption column connected to a membrane bioreactor to remove MG as they form improved overall yield (11). A microemulsion system also has been used to hydrolyze TG to obtain 2-MG (12). Yields of up to 80% were obtained; however, contamination of the product with the surfactant was cited as a possible disadvantage.

Recently, there have been significant developments in the area of conducting enzymatic and nonenzymatic reactions in

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supercritical fluid media, as discussed in several reviews (13–15) and book chapters (16,17). In addition to being environmentally benign, supercritical carbon dioxide (SC-CO$_2$) offers some unique advantages as a reaction medium. Faster reaction rates can be obtained due to the higher diffusion rates of solutes in SC-CO$_2$ and the lower viscosities exhibited by supercritical fluids, compared to reactions conducted in organic solvents. Resultant products also can be separated by controlling pressure, thus accelerating production of the desired products.

Numerous patents have been issued for glycerolysis processes. For example, glycerolysis of coconut oil at 240°C and 4.6 MPa with glycerol, containing 5–10% water, has been achieved, yielding a product that contains 56% MG and 12.7% FFA (U.S. Patent 2,474,740, cited in Ref. 1). An Indian patent (18), issued in 1962, describing a process of conducting glycerolysis in the presence of CO$_2$, claimed that a product was formed with 91% MG, when the reaction was carried out at 4.8–5.2 MPa, 240–250°C, from castor oil, after two hours. Further, it was demonstrated that the conversion to MG was higher in CO$_2$ than in air or nitrogen. The authors (18) also reported an increase in the conversion rate with pressure, over a 0.3–5.2 MPa pressure range. Even though such a process offers numerous advantages over conventional alkali-catalyzed glycerolysis, it would appear that it has not been utilized over the last three decades since its issuance. Thus, the objectives of this study were to investigate the effects of temperature, and particularly pressure, glycerol/oil ratio, and water concentration on the glycerolysis of soybean oil in SC-CO$_2$, and to compare conversion rates of different vegetable oils under optimum reaction conditions.

MATERIALS AND METHODS

Refined, bleached, and deodorized soybean, peanut, corn, canola, and cottonseed oils were purchased from P.V.O. Foods (Jacksonville, IL). Mono-, di-, and trilinolein and oleic acid standards and glycerol were purchased from Sigma Chemical Co. (St. Louis, MO). Welding-grade CO$_2$ (National Welding Supply, Bloomington, IL) was used for all experiments. Distilled water was added to the stirred reactor.

Glycerolysis reaction. Glycerolysis reactions were conducted in a 300-mL stirred autoclave (Magnedrive; Autoclave Engineers, Erie, PA), modified as shown in Figure 1. Soybean oil was used in optimizing the reaction conditions. Soybean oil, glycerol, and water were inserted into the autoclave in the desired ratios. The total amount of the reaction mixture was between 110–115 g, and occupied one-third of the cell volume. The reactor cell was initially filled with CO$_2$ and purged for 30 min to remove any air while stirring at 182 rpm. Then, the reactor was heated to the desired temperature, which was recorded by a Type J thermocouple located at the bottom of the cell, and maintained to ±1°C by a controller (Model CN4601-TR; Omega Engineering, Inc., Stamford, CT) . The system was pressurized by a gas booster pump (Model AGT-62/152; Haskel Inc., Burbank, CA), and the agitation speed was increased up to 1,040 rpm. The reaction was continued for four hours, and a ~2-mL sample of reaction mixture was removed through the sampling valve every 30 min for analysis. Samples were collected into 15-mL graduated centrifuge tubes sitting in ice to cool the hot reaction mixture immediately after removal from the autoclave. At the end of four hours, cold water was circulated into the autoclave jacket.
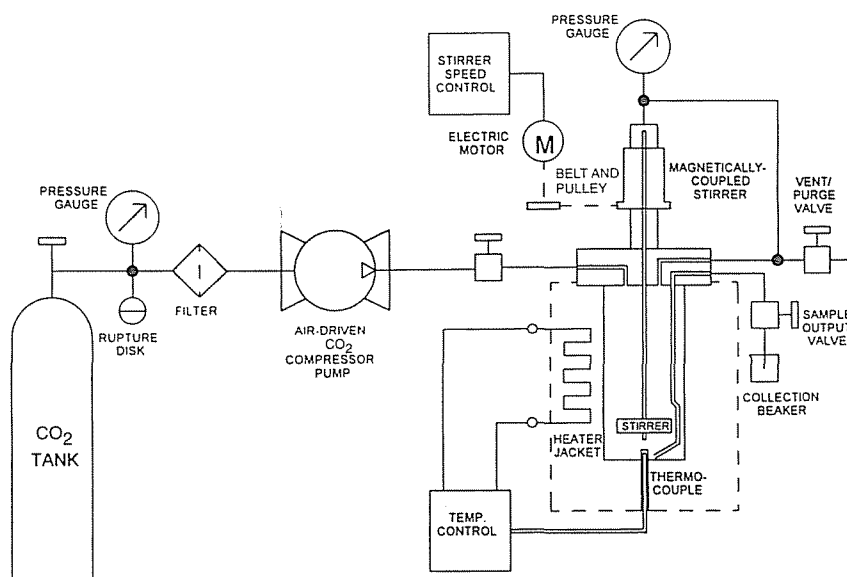

FIG. 1. Schematic of the stirred reaction system used for glycerolysis of oils in supercritical-carbon dioxide.

The mixture was cooled to below 60°C in approximately 10 min. After reaching room temperature, the reaction product was removed through the sampling valve while depressurizing the cell. Samples were centrifuged for 5 min at 3000 rpm to separate the oil and glycerol. The composition of the oil layer was then analyzed.

Analysis of reaction products. MG, DG, TG, and FFA content of the reaction samples were analyzed with a Lee Scientific Series 600 SFC/GC supercritical fluid chromatograph (SFC) (Dionex, Inc., Salt Lake City, UT). Docosane ($C_{22}H_{46}$) was used as the internal standard. About 10 mg of docosane and 100–110 mg of the oil product were dissolved in 10 mL petroleum ether/ethyl ether mixture (50:50, vol/vol) for injection into the SFC. The SFC unit was equipped with a timed injector (200 nL injection loop; Valco, Inc., Houston, TX), which was held open for 1.8 s; a Dionex SB-Octyl-50 capillary column (10 m × 100 µm i.d., 0.5 µm film thickness); a flame-ionization detector (FID) operating at 350°C; and an integrator (Data Jet-CH2; Spectra-Physics, San Jose, CA). The column temperature was held at 100°C for five minutes and then programmed to increase to 190°C at a rate of 8°C/min. The carrier gas was CO_2 (SFC/supercritical fluid extraction grade; Air Products, Allentown, PA); pressure was held at 12.2 MPa (120 atm) for five minutes, followed by an increase to 30.4 MPa (300 atm) at a rate of 0.8 MPa/min (8 atm/min) and a final hold of three minutes. Response factors for docosane, mono-, di-, and trilinolein were determined by running triplicate injections of 5, 10, 15, and 20 mg/mL standard solutions for which the correlation coefficients to the calibration curves were 0.9899, 0.9905, 0.9776, and 0.9589, respectively. Oleic acid was used to determine the FID response of FFA. Internal standard calculations were performed to calculate weight percentages of FFA, MG, DG, and TG fractions in the oil phase of the reaction mixture. Weight percentages were normalized to total 100% for every sample.

Experimental design. Glycerolysis reactions of soybean oil were conducted at temperatures of 150, 200, 250°C, and pressures of 20.7, 41.4, 62.1 MPa, glycerol/oil molar ratios of 15, 20, 25, and water concentrations of two and four percentage (wt% of glycerol). An average molecular weight of 870 a.m.u. was used for soybean oil to calculate molar ratios (19). A partial factorial design ($3^3 2^1$) of 18 experiments was conducted in random order. Based on these results, a second set of experiments was performed at a constant temperature of 250°C. This was a full factorial experimental design ($3^2 2^1$) of 18 randomized experiments, consisting of the three levels of pressure and glycerol/oil ratio and two levels of water concentration.

To study the effect of water concentration, soybean oil glycerolysis was carried out at 250°C, 20.7 MPa, and glycerol/oil ratio of 25 at water levels of 0–8%. The effect of SC-CO_2 was compared to the reactions run under subcritical conditions, 5.5 MPa, at 250°C, glycerol/oil ratio of 25 and 4% H_2O. Glycerolysis of peanut, corn, canola, and cottonseed oils were conducted at 250°C, 20.7 MPa, glycerol/oil ratio of 25, and 4% H_2O.

Analysis of variance of the results was performed by using

General Linear Model procedure of SAS Statistical Software, Version 6 (20). Means were compared by paired *t*-test of least square means at $\alpha = 0.05$.

RESULTS AND DISCUSSION

A typical SFC chromatogram of the reaction mixture from soybean oil glycerolysis is given in Figure 2. FFA, MG, DG, and TG had respective retention times of 11.0–12.9, 13.1–16.0, 18.5–21.5, and 22.8–27 min under the previously
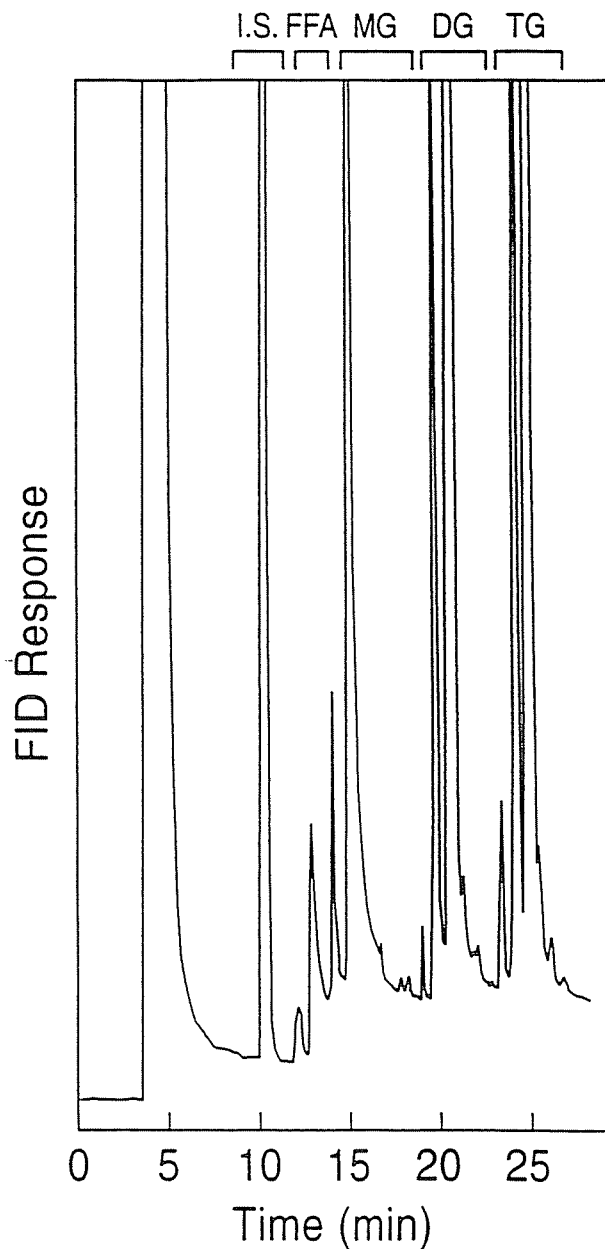

FIG. 2. Supercritical fluid chromatogram of a glycerolysis reaction product mixture; FID, flame-ionization detector; FFA, free fatty acids; MG, monoglycerides; DG, diglycerides; TG, triglycerides; I.S., internal standard.

described SFC program. Figure 3 shows the change in the composition of the soybean oil phase of the reaction mixture as the reaction proceeds at 250°C, 20.7 MPa, glycerol/oil ratio of 25, and 2% H_2O, which was typical for all reaction conditions studied. The TG concentration showed an exponential decrease with time. DG concentration increased, reached a maximum, and then started to decline as it was converted to MG. MG consistently increased with time. This behavior is typical for consecutive reactions going through the intermediate steps of $TG \rightleftharpoons DG \rightleftharpoons MG$. In the presence of water, hydrolysis occurred in concert with the glycerolysis, resulting in the formation of FFA. FFA initially increased then showed a slight decrease with time as FFA reacted with excess glycerol to form MG.

Effect of temperature. Glycerolysis did not occur at 150°C, regardless of the level of the other parameters studied, because no MG or DG could be detected by SFC analysis. When the temperature was increased to 200°C, there was still no MG formation; however, DG was synthesized at a level of 0.3–2.18%. No FFA formation was observed at 150 or 200°C. A further increase in the reaction temperature to 250°C resulted in a substantial conversion of TG into MG and DG. This was consistent with the fact that a high temperature is necessary to increase the solubility of glycerol in oil for glycerolysis to occur as described for the conventional process. Despite the high pressures used in this study, temperature was still the most critical factor to achieve conversion of TG. Thus, the remaining studies were conducted at 250°C.

Effect of glycerol/oil ratio. MG concentration, obtained at 250°C and 2% H_2O, is presented in Figure 4 as a function of glycerol/oil ratio and pressure. As expected, there was an in-

TABLE 1
Effect of Pressure on the Mean Values of MG, DG, TG, and FFA Compositions Obtained from Soybean Oil at 250°C[a]

Pressure (MPa)	Mean wt%			
	MG	DG	TG	FFA
20.7	46.0[a]	30.5[a]	12.9[c]	10.5[a]
41.4	12.4[b]	29.8[a]	51.4[b]	6.3[b]
62.1	3.1[c]	15.7[b]	77.4[a]	4.3[b]

[a]Means in a column with different superscripts are different at $P < 0.05$. MG, monoglycerides; DG, diglycerides; TG, triglycerides; FFA, free fatty acids.

crease in MG formation with an increase in the glycerol/oil ratio. Even though it was not statistically significant ($P > 0.05$), this increase was substantial at 20.7 MPa, which seems to be suppressed with an increase in pressure. Because this is a reversible reaction, an excess of glycerol results in the displacement of the equilibrium. Unreacted excess glycerol needs to be removed from the reaction mixture and recycled.

Effect of pressure. The effect of pressure on reaction rates is quite complex because pressure affects concentrations of reactants and products in solution, as well as mass transfer and the reaction rate constant itself. Pressure has favorable effects on the reaction rate constant if the activation volume is negative (16).

The pressure effect on the concentrations of MG, DG, TG, and FFA was significant ($P < 0.05$). Means of the concentrations of these components at the different pressure levels are presented in Table 1. MG formation decreased significantly ($P < 0.05$) with pressure (Fig. 4). This is in part due to the differences in the solubilities of these components in SC-CO_2. Unfortunately, it was not possible to visually observe the con-
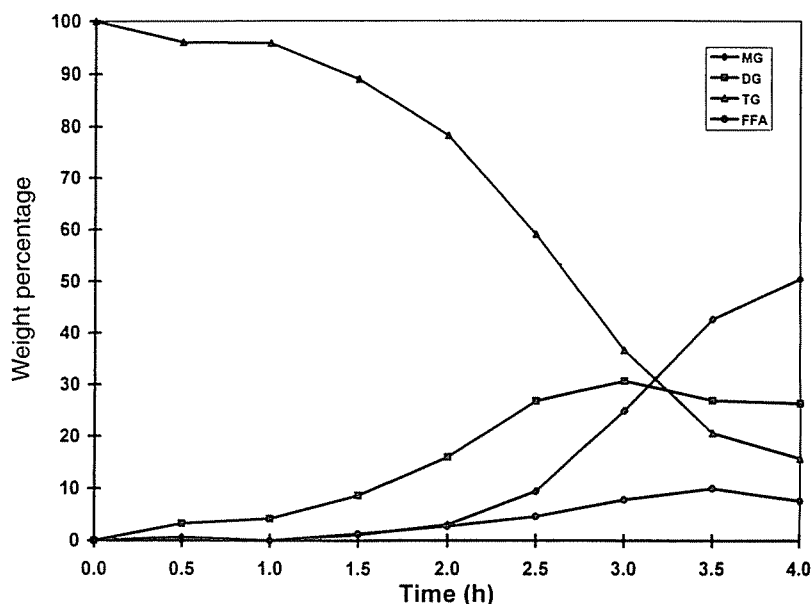

FIG. 3. Composition of the oil phase of the glycerolysis reaction mixture as a function of time at 250°C, 20.7 MPa, glycerol/oil ratio = 25, and 2% H_2O added to glycerol. See Figure 2 for abbreviations.
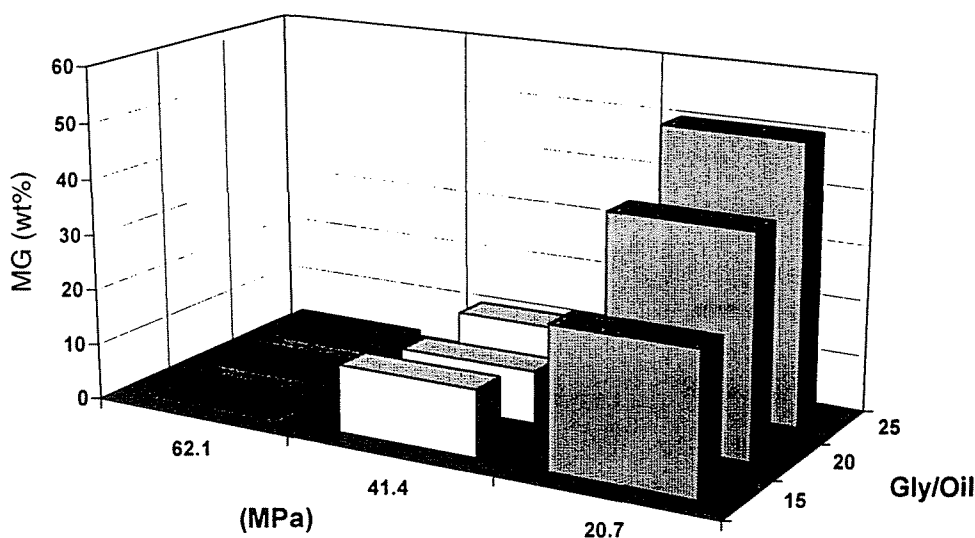
FIG. 4. MG content after four hours of glycerolysis in SC-CO_2 at 250°C and 2% H_2O added to glycerol as a function of pressure and glycerol/soybean oil (gly/oil) ratio. See Figure 2 for abbreviations.

tents of the reaction vessel, and there are no data available on their phase behavior at such high temperatures. Soybean oil TG become infinitely soluble in SC-CO_2 at 80°C and 95.0 MPa, due to the similarity in the oil and SC-CO_2 solubility parameters (21). Solubility studies on similar pure components have shown that the solubility of trilaurin was greater than that of dilaurin, which in turn was greater than that of monolaurin at 40°C and pressures above 22 MPa (22). This trend is due to an increase in the polarity of the components as the number of fatty acids esterified decreases, despite the decrease in the molecular weight. There are limited solubility data for glycerol in SC-CO_2 (23). Even though the molecular weight of glycerol is much lower than that of glycerides, its polar nature leads to low solubilities in SC-CO_2. With an increase in pressure, the solubility of TG in SC-CO_2 is increased to a greater extent than that of glycerol. Thus, the TG would be in the supercritical phase and not be available to react with glycerol in the liquid phase. In addition, the solubility of glycerol in the oil increases throughout the course of the reaction, as MG are formed, which act as emulsifiers between the two phases.

Phase equilibrium data for the H_2O–CO_2 system show approximately a fivefold increase in the concentration of water in SC-CO_2 at 20 MPa with an increase in temperature from 50 to 250°C, reaching ~35% (mole %) in the CO_2-rich phase (24). Similarly, solubilities reported for TG, DG, and MG in the above studies (21,22) also would be significantly increased at 250°C. However, due to the differences in the polarity and molecular weights of the components involved, their partitioning between the two phases to varying extents would be anticipated. Erickson *et al.* (25) also reported a significant decrease in the lipase-catalyzed acidolysis rate between palmitic acid and trilaurin when pressure was increased up to 30 MPa. They demonstrated that the decreased conver-

sion rate was not due to changes in the enzyme but was due to reactant partitioning between the supercritical phase and the enzyme.

Conversion rates achieved in the SC-CO_2 were also compared to those in subcritical CO_2 (Fig. 5). There was a 17% increase in the MG concentration when the pressure was increased from 5.5 to 20.7 MPa. However, a further increase in pressure resulted in a decrease in conversion. The FFA concentration of the products followed a similar trend to MG with pressure.

Effect of water. An increase in the water concentration from 2 (Fig. 4) to 4% (Fig. 6) resulted in similar trends with respect to pressure and glycerol/oil ratio. Conversion of TG to MG and DG increased with water, even though it did not reach statistically significant levels ($P = 0.09$). A maximum MG concentration of 49.2% was achieved at 20.7 MPa, a glycerol/oil ratio of 25 and 4% H_2O after 4 h. Figure 7 shows the effect of water on MG formation at 250°C, 20.7 MPa, and a glycerol/oil ratio of 25. Conversion of TG into MG doubled with 2% addition of water and did not show a substantial increase with a further increase in water. Even though the reaction is reported to be "additive," as in direct esterification, so that no water is absorbed or eliminated (1), the presence of optimal levels of water did improve the reaction rate. Dey *et al.* (cited in Ref. 3) reported an increase in the yield of MG when 4–5% water was added to glycerol during glycerolysis of castor oil at 250°C and 1.4 MPa of CO_2. Ross *et al.* (26) also indicated that water can act as a catalyst when studying glycerolysis of coconut oil. Yamane *et al.* (4) concluded that water concentration should be below 4% in glycerol to minimize FFA formation during lipase-catalyzed glycerolysis of corn oil. McNeill *et al.* (27) also showed a reduction in MG yields at water concentrations above 8% in glycerol during enzymatic glycerolysis of beef tallow.
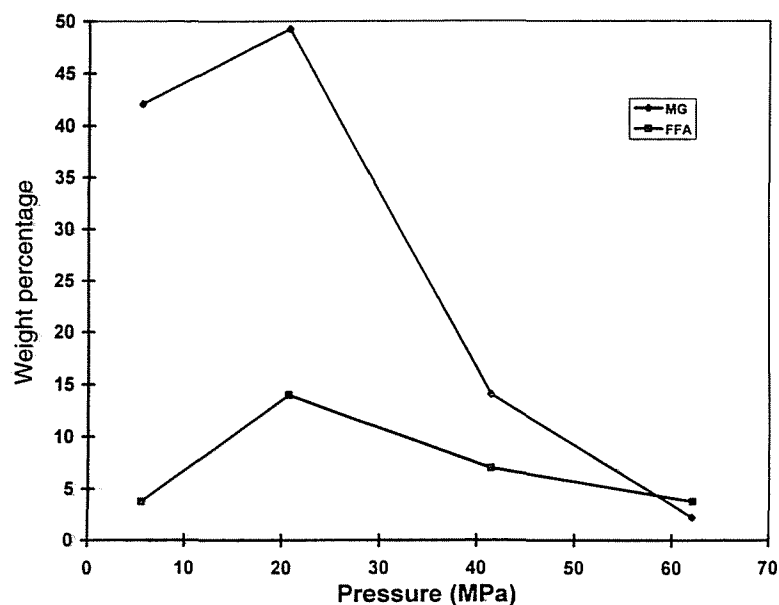
FIG. 5. Effect of pressure on MG formation in SC-CO_2 at 250°C, glycerol/soybean oil ratio of 25, and 4% H_2O added to glycerol. See Figure 2 for abbreviations.

The effect of water concentration on FFA formation was significant ($P < 0.05$). As shown in Figure 7, there is FFA formation even without the addition of water to the reaction mixture. This may be due to the presence of water in the glycerol starting material, which was subsequently determined to be 4%. FFA concentration showed a steady increase with water addition up to 6%, reaching 16.0%, and remained steady with a further increase in water concentration. A water level of 2% (wt% of glycerol) corresponded to a glycerol/water molar ratio of 9.8, which decreased to 2.4 at a level of 8%. In the presence of water, it is evident that hydrolysis and glyceroly-

sis reactions are taking place simultaneously. Water and glycerol have opposing effects on the equilibrium; excess water shifts the equilibrium toward the formation of FFA by hydrolyzing the ester linkages, while excess glycerol shifts the equilibrium in the opposite direction toward the formation of MG. At a 2% water level, glycerol is more dominant, suppressing the effect of water; however, at higher water levels, the hydrolysis reaction becomes significant, leading to an increase in FFA.

Effect of the type of oil. Composition of the glycerolysis products of the different vegetable oils, obtained at 250°C,
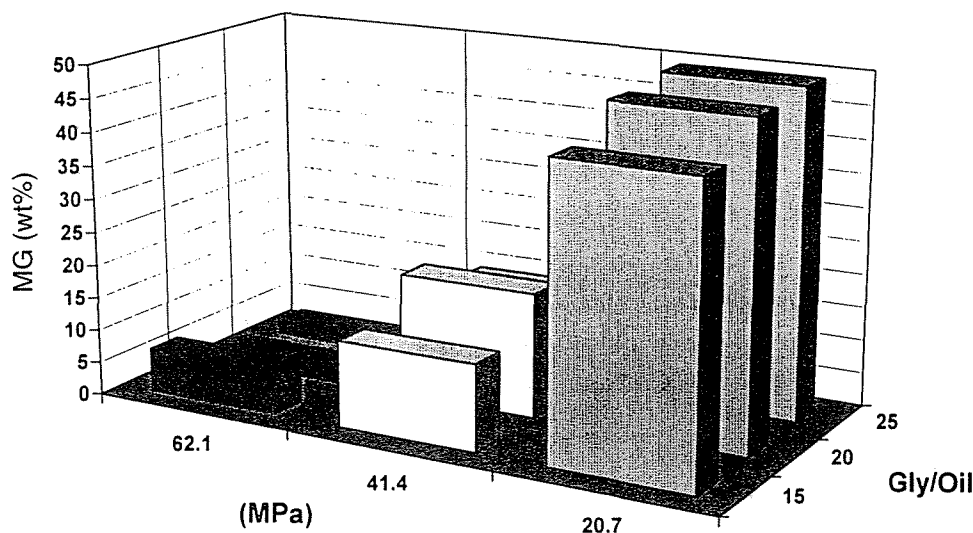

FIG. 6. MG content after four hours of glycerolysis in supercritical-carbon dioxide at 250°C and 4% H_2O added to glycerol as a function of pressure and glycerol/soybean oil (gly/oil) ratio. See Figure 2 for abbreviations.
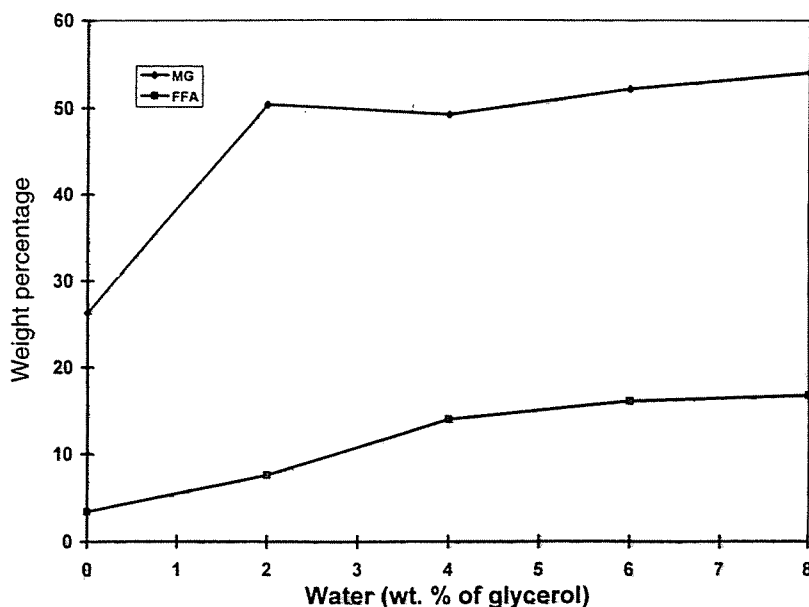
FIG. 7. Effect of water concentration on MG formation in supercritical-carbon dioxide at 250°C, 20.7 MPa, and glycerol/soybean oil ratio of 25. See Figure 2 for abbreviations.

20.7 MPa, glycerol/oil ratio of 25, and 4% H_2O, are given in Table 2. There were slight differences in the composition of the products, and the level of MG formation was highest with soybean oil and lowest for cottonseed oil. The solubility of glycerol at 250°C is reported to vary from 40–45% for hydrogenated cottonseed oil to 65% for coconut oil (1), depending on the fatty acid composition of the oil. Such a variation in solubility could be responsible for the differences in conversion rates achieved for the various oils.

Effect of cooling rate and agitation. Cooling rates at the end of the 4-h reaction time were conducted fairly rapidly. In these studies, the reaction mixture was cooled down to below 60°C from 250°C in approximately 10 min. In general, reversion was minimal or not observed at all. On the contrary, MG concentration continued to increase during the cooling period under some conditions. However, at the higher moisture levels, a decrease in the MG concentration as high as 10% was measured. In conventional glycerolysis, complete neutralization of the alkali catalyst, prior to cooling of the reaction mixture, is crucial to minimize reversion, which can be as high as 30% in incomplete neutralization (1). The catalyst is neutral-

ized by the addition of phosphoric acid, followed by adsorption of neutralization products with clays, which leads to a loss of MG.

Agitation is another important parameter that affects conversion rates. Yamane *et al.* (4) showed that conversion rate increased with agitation speed, reached a maximum at 350 rpm, and remained constant at agitation speeds above this value. Superemulsification effects, discussed by Sonntag (1), may be present in this study due to the high agitation rates used (1040 rpm). However, it was not possible to measure liquid droplet size in the $SC-CO_2$ medium. The yield of MG cannot be explained by the solubility of glycerol in oil alone because conditions of superemulsification also could contribute to reaction in the heterogeneous phases. The fact that supercritical fluids have negligible surface tension should increase the contact between the phases involved in the reaction, contributing to potentially higher conversion rates.

In conclusion, this study demonstrated the potential for conducting glycerolysis reactions in $SC-CO_2$ atmosphere. $SC-CO_2$ offers the advantage of eliminating the need for conventional alkali catalyst and its neutralization at the end of the reaction with the attendant loss of MG through reversion and filtration. CO_2 can easily be removed from the reaction mixture *via* release of pressure. Conversion rates obtained in this study were better than those obtained in conventional glycerolysis. The products obtained were lighter in color, and free from undesirable odors, because the CO_2 atmosphere minimizes any chance of oxidation. Although the described reaction requires high-pressure equipment, the operation is considerably simpler than conventional alkali-catalyzed glycerolysis, off-setting some of the expense attendant with the construction of a high-pressure plant. Finally, supercritical technology offers the possibility of developing a continuous

TABLE 2
Composition (wt%) of Glycerolysis Products from Different Vegetable Oils Obtained at 250°C, 20.7 MPa, Glycerol/Oil Ratio of 25, and 4% Water after 4-h Reaction Time[a]

Type of oil	MG	DG	TG	FFA
Soybean	49.2	26.6	10.1	14.0
Peanut	46.6	32.1	12.5	8.8
Cottonseed	41.1	35.0	12.6	11.3
Corn	45.6	32.3	13.0	9.2
Canola	41.7	33.0	16.0	9.3

[a]See Table 1 for abbreviations.

simultaneous extraction–reaction–fractionation process, where extraction of the oil with SC-CO_2 from an oilseed matrix can be conducted in the first step, followed by conversion to MG in the reaction stage and eventually fractionation/enrichment of the MG from the resultant synthesized mixture.

ACKNOWLEDGMENTS

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